

IN THE CLAIMS:

Please amend the claims to read as set forth in the Listing of Claims below:

1. (currently amended) A method for isolating preparing fetal nucleated red blood cells (NRBCs) present in maternal peripheral blood for prenatal genetic investigation, comprising the steps of:

a) transferring mixing maternal blood into non physiological, tissue culture medium, which after addition of and an aqueous solution containing citric acid, Na citrate and dextran, to form a non-physiological tissue culture mixture has having the following characteristics:

| | | |
|------------------|-----------|--------|
| pH | 6.4-6.6 | |
| osmolality | 300-330 | mOsm |
| Na ⁺ | 150-170 | mmol/l |
| K ⁺ | 4.5-5.5 | mmol/l |
| Cl ⁻ | 100-115 | mmol/l |
| Ca ⁺⁺ | 1.00-2.50 | mmol/l |
| glucose | 400-500 | mg/dl |
| Lactate | 10-20 | mg/dl |

b) maternal blood, as modified in a) is transferred transferring the non-physiological tissue culture mixture obtained in step a) into a cell separation device, followed by the introduction introducing into the said separation device of a liquid having an higher a density higher than maternal blood and containing a red blood cell RBCs aggregating agent,

c) the nucleated cells, having a lower density than the liquid introduced in the step b) are isolated; in the discontinuous density gradient, by subjecting the separation device to centrifugal force to isolate the NRBCs having a lower density than the liquid introduced in step b);

d) washing the isolated cells NRBCs are washed and resuspended resuspending them in tissue culture medium to regain physiological cell metabolism; and

e) identifying fetal eells are identified NRBCs by appropriate identification procedures and eounted counting said fetal NRBCs.

2. (cancelled).

3. (currently amended) The method of claim 3 1 in which the non-physiological medium mixture obtained in step a) has the following characteristics:

| | | |
|------------|-----|------|
| pH | 6.5 | |
| osmolality | 320 | mOsm |

| | | |
|------------------|------|--------|
| Na ⁺ | 165 | mmol/l |
| K ⁺ | 5.35 | mmol/l |
| Cl ⁻ | 110 | mmol/l |
| Ca ⁺⁺ | 1.25 | mmol/l |
| glucose | 500 | mg/dl |
| lactate | 10 | mg/dl; |

4. (original) The method of claim 1 in which the RBCs aggregating agent of step b) is Ficoll.

5. (original) The method of claim 1 in which the density of the liquid introduced in the separation device by the step b) is 1.068 g/ml.

6. (currently amended) The method of claim 1 in which the separation device used in step b), comprises an elongated chamber (1), whose cross section decreases from the base towards the top, at least a first channel (2) one end of which opens into the said chamber near the said base and the other end is connected to a pressurized liquid source, and a second channel (3) one end of which opens into the same elongated chamber (1) at the device top while the other end opens at the exterior of the device, the said device further comprising at least one additional channel (4), one end of which opens at a middle level of said chamber height and the other end opens at the exterior of the device.

7. (new) A method for identifying fetal nucleated red blood cells (NRBCs) present in maternal peripheral blood, comprising the steps of:

a) mixing maternal blood, tissue culture medium, and an aqueous solution containing citric acid, Na citrate and dextran, to form a non-physiological tissue culture mixture having the following characteristics:

| | | |
|------------------|-----------|--------|
| pH | 6.4-6.6 | |
| osmolality | 300-330 | mOsm |
| Na ⁺ | 150-170 | mmol/l |
| K ⁺ | 4.5-5.5 | mmol/l |
| Cl ⁻ | 100-115 | mmol/l |
| Ca ⁺⁺ | 1.00-2.50 | mmol/l |
| glucose | 400-500 | mg/dl |
| lactate | 10-20 | mg/dl; |

b) transferring the non-physiological tissue culture mixture obtained in a) into a cell separation device, followed by introducing into said separation device a liquid

having a density higher than the red blood cells (RBCs) and containing a red blood cell (RBC) aggregating agent;

c) in discontinuous density gradient, subjecting the separation device to centrifugal force to isolate the NRBCs having a lower density than the liquid introduced in step b);

d) washing the isolated NRBCs and resuspending them in tissue culture medium; and

c) identifying by appropriate identification procedures fetal NRBCs.

8. (new) The method of claim 7, further comprising counting the identified fetal NRBCs.

9. (new) The method of claim 1, in which the separation device is as illustrated in Fig. 1.